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[Note]

Discovery of the large narcissus fly, *Merodon equestris* (Fabricius), (Diptera, Syrphidae) in South Korea

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Abstract

We found the large narcissus fly, *Merodon equestris* (Fabricius), which has been doubted to occur in Korea. This species is an economically important in management of narcissus and also of quarantine pests. We therefore provide the morphological diagnosis and DNA barcode sequences for rapid species identification of *M. equestris* based on the five Korean specimens.

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Introduction

Merodon equestris is well known as "the large narcissus fly", which is a major pest of narcissus (Liliaceae) at larval stage (Hodson, 1932; Rotheray, 1994; Marcos-García *et al.,* 2011). This species is currently recognized to distribute in Holarctic. However, the original range is believed to be southern Europe. And then, its range has almost certainly been expanded within Europe since the late of 19th century and has become widespread to other geographical ranges including Japan, North America and New Zealand due to human activity (Woodville, 1970; Speight, 2011).

In Korea, the members belonging to the genus *Merodon* Meigen have been recorded as two species: *Merodon equestris* (Fabricius) and *M. kawamurai* Matsumura. However, between them, *M. equestris* has been recently doubted about its Korean distribution due to the fact that many earlier Korean records of this species showed misidentifications of *Mallota* species (especially for *Mallota tricolor* Loew) and any Korean specimens has not been available (Han and Choi, 2001; Han *et al.,* 2014).

However, we found *M. equestris* from the Korean specimens in process of construction of DNA barcode library focused on the Korean insects. The species was initially recognized throughout comparison of DNA barcoding region of *COI* (*cytochrome c oxidase subunit I*) gene of unidentified syrphid specimens using BLAST search in NCBI, and then reconfirmed it morphologically. Such process indicates that construction of the DNA barcode library from randomly collected insects in a local fauna allow laymen on taxonomy of Syrphidae to find out economically important species in a rapid and easy manner and/or quarantine insect pests.

We herein report the presence of *M. equestris* in Korea based on morphology and DNA barcode data.

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Materials and Methods

Sample collection, DNA barcoding analysis and identification

Five specimens of *Merodon equestris* were collected from South Korea. The specimens were caught alive on a botanical garden sited in Yongin-si, Gyeonggi-do province and were individually stored at -20°C to maintain DNA friendly condition until DNA barcoding analysis. The protocol of DNA barcoding analysis was followed by our previous studies (e.g., Han *et al.,* 2016; 2018). For molecular species identification of our *COI* sequence data, we used BLAST search tool in MEGA 5.2 (Tamura *et al.,* 2011) and added the identical and highly similar 27 *COI* sequences of two species, *M. equestris*, *M. confuses* Marcos-Gracía, Vujić, Ricarte and Ståhls, and a *COI* sequence of *M. albifrons* Meigen to use as outgroup from GenBank (http://www.ncbi.nlm.nih.gov/genbank) (Marcos-Gracía *et al.,* 2011; Penny *et al.,* 2012; Menqual *et al.,* 2015; Hebert *et al.,* 2016; Dewaard, 2017, unpublished). For morphological examination, the general features of the specimens were observed under a stereoscopic microscope (MZ 16A and MZ 6; Leica, Solms, Germany). The species identification was determined according to the previous taxonomic works (Marcos-Gracía *et al.,* 2007; 2011). All of the examined materials and genomic DNA stocks have been preserved in the insect collection at the National Institute of Agricultural Science (NIAS), Jeonju, Korea. The five COI sequences generated in this study are available in GenBank under accession number MH383061–MH383065.

Results

COI profile

We successfully obtained five *COI* sequences from the five Korean specimens. Our final combined dataset consisted of 34 *COI* sequences from three *Merodon* species including an outgroup (Table 1; Supplementary Table S1). There was no evidence of pseudogenes or heteroplasmy. We found 609 conserved sites (92.6%) and 49 variable sites (7.4%), of which 18 (2.7%) were parsimoniously informative sites and 31 (4.7%) were singleton sites. The base composition of the *COI* sequences of all taxa was significantly biased toward TA (70.6%).

The neighbor-joining (NJ) tree (Fig. 1) showed that Clade A is composed of *M. equestris* including the five Korean specimens with low intraspecific genetic distances (range: 0–1.9%) and Clade B is composed of *M. confuses* and a *COI* sequences of *M. equestris* (MG1663351) with more variable intraspecific genetic distances (range: 0–2.5%). Two clades (A and B) were separated by 1.0 to 4.0% of interspecific genetic distances. The five Korean specimens could be identified as *M. equestris* by DNA taxonomy in this study.

Systematic accounts

Family Syrphidae Latreille, 1802 꽃등에과
Subfamily Milesiinae Bezzi, 1893 알락긴꽃등에과
Tribe Eumerini Smirnov, 1924
Merodon equestris (Fabricius, 1794) 수선화꽃등에 (Fig. 2–3)
Syrphus equestris Fabricius, 1794: 292.
Eristalis ferrugineus Fabricius, 1905: 240.
Eristalis narcissi Fabricius, 1805: 239.
Merodon bulborum Rondani, 1845: 256
Merodon constans Wiedemann, 1822: 354.
Merodon nigrithorax Bezzi, 1900: 89.
Merodon nobilis Meigen, 1822: 353.
Merodon transversalis Wiedemann, 1822: 354.

Table 1. Summary of the *Merodon* species examined in this study.

Species ID number	Species	Sample size	Collected localities	Source of *COI* sequences	
				Present study	NCBI
1	*Merodon equestris* (Fabricius, 1794)	25	Korea (5), Canada (13), Finland (1), Spain (2), Turkey (3)	○	○
2	Merodon confusus Marcos-Gracía, Vujić, Ricarte and Ståhls, 2011	8	Spain (8)		○
3†	*Merodon albifrons* Meigen, 1822	1	Greece (1)		○

† indicates an outgroup taxon.

Supplementary Table S1. List of 34 *COI* sequences of 11 species of the genus *Merodon* in this study.

No. of indis.	Species	Voucher nos	Locality	Date collected	Preserved conditions	GenBank Accession No.	References
1	*Merodon equestris* (Fabricius, 1794)	6402	Korea: Gyeonggi (GG), Yongin-si, Baekam-myeon, Oksan-ri, Hantaeck Botanical garden	1. VI. 2012	Frozen	MH383061	This study
2	*Merodon equestris* (Fabricius, 1794)	6403	Korea: Gyeonggi (GG), Yongin-si, Baekam-myeon, Oksan-ri, Hantaeck Botanical garden	1. VI. 2012	Frozen	MH383062	This study
3	*Merodon equestris* (Fabricius, 1794)	6410	Korea: Gyeonggi (GG), Yongin-si, Baekam-myeon, Oksan-ri, Hantaeck Botanical garden	1. VI. 2012	Frozen	MH383063	This study
4	*Merodon equestris* (Fabricius, 1794)	6413	Korea: Gyeonggi (GG), Yongin-si, Baekam-myeon, Oksan-ri, Hantaeck Botanical garden	1. VI. 2012	Frozen	MH383064	This study
5	*Merodon equestris* (Fabricius, 1794)	6458	Korea: Gyeonggi (GG), Yongin-si, Baekam-myeon, Oksan-ri, Hantaeck Botanical garden	1. VI. 2012	Frozen	MH383065	This study
6	*Merodon equestris* (Fabricius, 1794)	BIOUG01427-E09	Canada			MG170543	Dewaard (2017, unpublished)
7	*Merodon equestris* (Fabricius, 1794)	BIOUG01427-H09	Canada			MG169358	Dewaard (2017, unpublished)
8	*Merodon equestris* (Fabricius, 1794)	BIOUG01427-E11	Canada			MG164389	Dewaard (2017, unpublished)
9	*Merodon equestris* (Fabricius, 1794)	BIOUG01427-F01	Canada			MG163351	Dewaard (2017, unpublished)
10	*Merodon equestris* (Fabricius, 1794)	BIOUG01427-H01	Canada			MG163224	Dewaard (2017, unpublished)
11	*Merodon equestris* (Fabricius, 1794)	BIOUG27671-D05	Canada			MG165657	Dewaard (2017, unpublished)
12	*Merodon equestris* (Fabricius, 1794)	BIOUG01427-F03	Canada			MG165657	Dewaard (2017, unpublished)
13	*Merodon equestris* (Fabricius, 1794)	BIOUG28571-D04	Canada			MG170000	Dewaard (2017, unpublished)
14	*Merodon equestris* (Fabricius, 1794)	BIOUG01427-E12	Canada			MG164422	Dewaard (2017, unpublished)
15	*Merodon equestris* (Fabricius, 1794)	BIOUG17754-A05	Canada			KR979911	Hebert et al. (2016)
16	*Merodon equestris* (Fabricius, 1794)	BIOUG00992-F06	Canada			KT111790	Hebert et al. (2016)
17	*Merodon equestris* (Fabricius, 1794)	BIOUG08409-C06	Canada			KR672399	Hebert et al. (2016)

Supplementary Table S1. Continued

No. of indis.	Species	Voucher nos	Locality	Date collected	Preserved conditions	GenBank Accession No.	References
18	*Merodon equestris* (Fabricius, 1794)	CNC: Diptera: 45690	Canada			JN992010	Penny et al. (2012)
19	*Merodon equestris* (Fabricius, 1794)	Y690_2007	Finland			EU431486	Menqual et al. (2015)
20	*Merodon equestris* (Fabricius, 1794)	MZH:Y753_2009	Spain			FR717716	Marcos-García et al. (2011)
21	*Merodon equestris* (Fabricius, 1794)	MZH:Y32_2004	Spain			FR717715	Marcos-García et al. (2011)
22	*Merodon equestris* (Fabricius, 1794)	MZH_Y837_2009	Turkey			FR717727	Marcos-García et al. (2011)
23	*Merodon equestris* (Fabricius, 1794)	MZH_Y836_2009	Turkey			FR717726	Marcos-García et al. (2011)
24	*Merodon equestris* (Fabricius, 1794)	MZH_Y835_2009	Turkey			FR717725	Marcos-García et al. (2011)
25	*Merodon equestris* (Fabricius, 1794)	MZH_Y834_2009	Turkey			FR717724	Marcos-García et al. (2011)
26	*Merodon confusus* Marcos-García, Vujić, Ricarte and Ståhls, 2011	MZH:4744_2009	Spain			FR717723	Marcos-García et al. (2011)
27	*Merodon confusus* Marcos-García, Vujić, Ricarte and Ståhls, 2011	MZH:4740_2009	Spain			FR717722	Marcos-García et al. (2011)
28	*Merodon confusus* Marcos-García, Vujić, Ricarte and Ståhls, 2011	MZH:4738_2009	Spain			FR717721	Marcos-García et al. (2011)
29	*Merodon confusus* Marcos-García, Vujić, Ricarte and Ståhls, 2011	MZH:4725_2009	Spain			FR717720	Marcos-García et al. (2011)
30	*Merodon confusus* Marcos-García, Vujić, Ricarte and Ståhls, 2011	MZH:4723_2009	Spain			FR717719	Marcos-García et al. (2011)
31	*Merodon confusus* Marcos-García, Vujić, Ricarte and Ståhls, 2011	MZH:4722_2009	Spain			FR717718	Marcos-García et al. (2011)
32	*Merodon confusus* Marcos-García, Vujić, Ricarte and Ståhls, 2011	MZH_S478_2005	Spain			FR717717	Marcos-García et al. (2011)
33	*Merodon confusus* Marcos-García, Vujić, Ricarte and Ståhls, 2011	MZH:S478_2005	Spain			FR717714	Marcos-García et al. (2011)
34†	*Merodon albifrons* Meigen, 1822	MZH: Y923	Greece			LN906863	Marcos-García et al. (2011)

† outgroup taxon.

Merodon tuberculatus Rondani, 1845: 256.

Merodon validus Wiedemann, 1822: 365.

Musca bombyliformis Geoffroy, 1785: 479.

Syrphus flavicans Fabricius, 1794: 292.

[Korean records] The following records are mostly misidentifications of *Mallota* spp. (see Han and Choi 2001; see also Han et al. 2014). ***Merodon equestris*:** Kim, JI 1980: 388 (Korean check.); ESK & KSAE 1994: 290 (Korean check.); Han et al. 1998: 135 (Korean cat.); Han & Choi 2001: 140 (Korean check.); Paek et al. 2010: 231 (Korean check.); Han et al. 2014: 20 (Korean check.). ***Lampetia equestris*:** Kim, CW 1971: 846 (redescrip.); Kim, JI 1975: 41 (Korean check.); Kim, CW 1980:

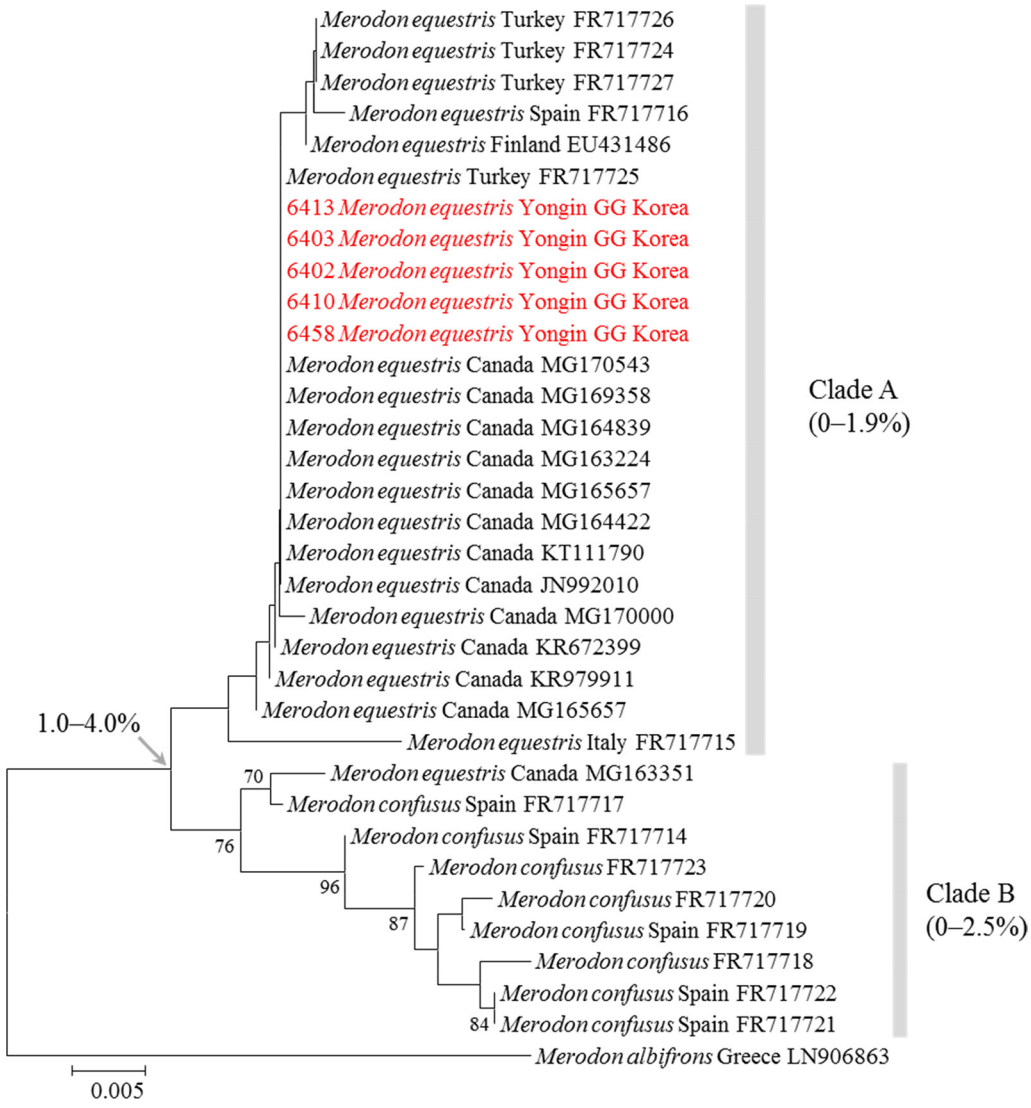

Fig. 1. Neighbor-joining tree inferred from partial *COI* gene sequences based on 34 specimens of *Merodon* spp. The percentage denotes the range of genetic distance.

278 (distr. Map).

Material examined. 3 males, 1 female, Hantaeck botanical garden, Oksan-ri, Baekam-myeon, Yongin-si, Gyeonggi-do, South Korea. 1. VI. 2012. Taeman Han; 1 male, ditto, Young Bo Lee.

Diagnosis. Body (Fig. 2) 13–15 mm in length, bumble bee-like species with densely covered body hairs; eyes large, contacted in center of anterior in male (Fig. 3A), but not in female (Fig. 3B); posterior part of midcoxa hairy, posterior anepisternum with reduced hairs; Hind tibia (Fig. 3C–D) with large central bulge at inner side, apical process of hind tibia long and conspicuously incurved in male, but simple in female. Male genitalia: posterior surstyle lobe (PSL) of epandrium with rounded top (Fig. 3E); surstyle margin slightly arched; cercus large, triangular; hypandrium (Fig. 3F) with folded thecal ridge (TR); lateral sclerite of aedeagus (LSA) prominent, oval shaped at apex.

Variability. The body color polymorphism of this species is known to be variable. The Korean specimens examined in this study can be represented into three color patterns regardless of sexes: 1) with almost completely pale yellow hairs (Fig. 2A–B); 2) with almost completely black hairs (Fig. 2C–D); 3) with black haired posterior half of scutellum and tergite III (Fig. 2E–F).

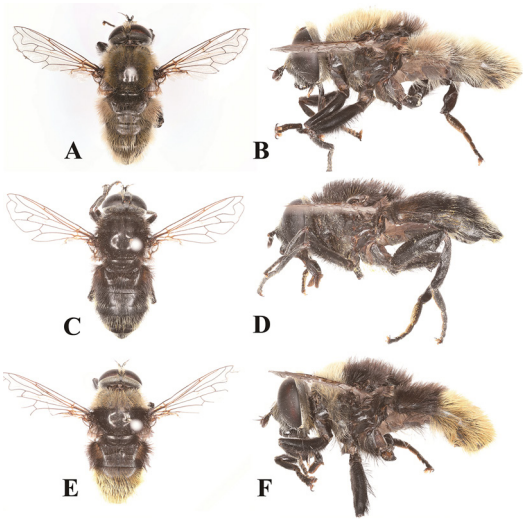

Fig. 2. *Merodon equestris* (Fabricius) from South Korea, adult color polymorphisms. A–B: almost completely pale yellow type (specimen no. 6403, male). C–D: almost wholly back type (specimen no. 6410, female). E–F: partially black type (from posterior half of scuteum and tergite III) (specimens no. 6413, male). A, C, E: dorsal view. B, D, F: lateral view.

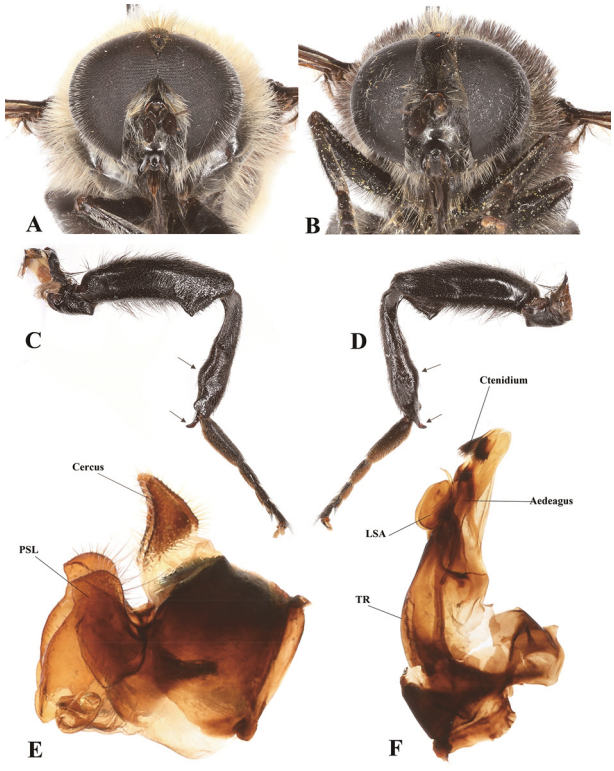

Fig. 3. *Merodon equestris* (Fabricius) from South Korea. A: head in anterior view (specimens no. 6403, male). B: ditto (specimens no. 6410, female). C: left hind leg at outside (specimens no. 6458, male). D: ditto at inner side. E. epandrium (specimen no. 6458, male). F. hypandrium (specimens no. 6458, male). PSL: posterior surstyle lobe. LSA: lateral sclerite of aedeagus.

Larva. Morphology (Hodson, 1932; Rotheray, 1994); internal feeder in tissues of bulbs of Liliaceae (Speight, 2011).

Notes. Kim (1971: 846) firstly reported *M. equestris* (as *Lampetia equestris*) with species diagnosis and an illustration based on a male specimen collected at Mt. Songri of middle of South Korea in 1957. He described as *"legs black, hind femur stout, hind tibia with central bulge, a long apical process at inner-side, and a plate-liked process at out-side"* This is similar to diagnostic characters of the hind leg of *M. equestris*, but not exactly corresponded with the species (see Diagnosis as above). Furthermore, the male illustration (pl. 51, Fig. 204) was drawn only the dorsal aspect of the male. It is considered to be difficult an exact species identification for *M. equestris* by lacking the crucial diagnostic characters of the hind leg. And the male illustration also closely resembles *Mallota tricolor*. Han and Choi (2001: 142) pointed out "the Korean distribution of *M. equestris* is doubted, except a possibility of introduced species from their nature range (such as Europe). There were many Korean records of this species but the almost of them were misidentified of *Mallota* spp., especially with *Mallota tricolor*. We consider these previous misidentifications may be caused by the male illustration by Kim (1971), which is more similar to *Mallota* species".

In this study, we could verify the presence of *M. equestris* in Korea based on the five Korean specimens using molecular and morphological identification. However, we could not determine whether the Korean specimens are a native in Korea or an invasive. Nonetheless, our study is expected to provide taxonomic information for rapid species identification of *M. equestris*, which is an important species for the management of narcissus and also for quarantine perspective by both molecular and morphological methods.

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